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## Original article

Value of conventional cytology in the presence of  
macroscopic lesions of the anal canal<sup>☆</sup>Lêda Pereira de Barcellos<sup>a,\*</sup>, Fábio Russomano<sup>b</sup>, José Ricardo Hildebrandt Coutinho<sup>a,c</sup><sup>a</sup>Hospital Federal de Ipanema M.S, Rio de Janeiro, RJ, Brazil<sup>b</sup>Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira (IFF/FIOCRUZ), Rio de Janeiro, RJ, Brazil<sup>c</sup>Instituto de Pesquisa Clínica Evandro Chagas (IPEC/FIOCRUZ), Rio de Janeiro, RJ, Brazil

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## ABSTRACT

**Objectives:** To verify the value of conventional cytology for the diagnosis of macroscopic lesions of the anal canal and to describe the limitations of the samples.

**Method:** We evaluated 395 conventional cytology samples obtained by brushing the anal canal of patients (predominantly male, HIV-positive) and compared them to the presence of macroscopic lesions of the anal canal observed under anorectal examination.

**Results:** Of the total, 91.6% of samples were classified as adequate. Cellular elements representative of the anal transformation zone were observed in 63.5% of samples. Sensitivity in the presence or absence of cellularity was 80% and 31%, respectively.

**Conclusion:** The study demonstrates the feasibility of using conventional anal cytology in outpatients.

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Este é um artigo Open Access sob a licença de [CC BY-NC-ND](http://creativecommons.org/licenses/by-nc-nd/4.0/)Valor da citologia convencional na presença de lesões macroscópicas do  
canal anal

## RESUMO

**Objetivo:** verificar o valor da citologia convencional no diagnóstico de lesões macroscópicas do canal anal e descrever as limitações das amostras obtidas.

**Método:** avaliamos 395 exames citológicos convencionais obtidos por escovado do canal anal de pacientes predominantemente do sexo masculino, soropositivos para HIV, e comparamos com a presença de lesões macroscópicas do canal anal constatadas ao exame proctológico.

**Resultado:** o percentual de amostras adequadas foi de 91,6%, e os elementos celulares representativos da zona de transformação anal foram observados em 63,5% das amostras. Encontramos sensibilidade de 80% e 31% na presença ou ausência desta celularidade, respectivamente.

## Palavras-chave:

HPV

Prevenção de neoplasia anal

Citologia convencional

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**Conclusão:** O estudo demonstra a possibilidade de utilização da citologia anal convencional no rastreamento de lesões macroscópicas do canal anal em pacientes ambulatoriais.

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## Introduction

The incidence of anal squamous cell carcinoma and its precursor lesions increased in recent decades, and it has been widely proven the participation of HPV (human papillomavirus) types 16 and 18 in its pathogenesis.<sup>1</sup>

Although still a relatively rare cancer, its incidence has been rising alarmingly in younger patients, especially men who practice receptive anal sex with men (MSM, so-called by its acronym in English), independent of HIV infection, but with greater involvement of those infected with this virus. Women with a history of multicentric squamous intraepithelial neoplasia, heterosexual men and women HIV-positive or immunosuppressed for other reasons also have contributed to the increased incidence of this neoplasia.<sup>2</sup>

In Brazil we do not have data on the incidence of anal cancer, because they are included in the colon and rectum topography. In these topographies 14,180 and 15,960 new cancer cases in men and in women, respectively, are expected, corresponding to an estimated risk of 15 new cases per 100,000 men and 16 new cases per 100,000 women.<sup>3</sup> Regarding the situation of AIDS, there has been an increase in the number of cases of the disease in young people included in the MSM exposure category.<sup>4</sup>

The highly active antiretroviral therapy (HAART) seems not to modify the pathophysiology of HPV anal lesion in HIV-infected patients, suggesting that a progression to serious injury should occur in patients with prolonged survival.<sup>5</sup>

The similarities between cervical and anal cancers, for instance, the association with HPV, the greater occurrence in the existing epithelial transformation zone in both topographies and the ability to diagnose early lesions susceptible to less aggressive treatments, led to the use of exfoliative cytology as a diagnostic test for more than a decade, and this procedure can be performed by the conventional method or in liquid medium, with similar results. Still, the current incidence of anal cancer is similar to that of cervical cancer before the establishment of prevention programs, bringing great expectations in the use of anal cytology.<sup>1,2</sup>

Several studies show that cytology has a good sensitivity but low specificity. A meta-analysis reported variations in sensitivity between 42 and 98% and of specificity between 16 and 96%.<sup>1</sup> An important factor for this variation may be due to lack of standardization of how the cytobrush should be introduced into the anal canal<sup>6</sup> and to the greater difficulty in the obtainment and preparation of anal canal samples, compared with the cervical collection.<sup>7</sup> The evaluation of the screening effectiveness for long-term prevention of anal as well as cervical cancer still remains to be done.<sup>1</sup> A contributing factor to this performance variation is the little experience of cytopathologists with the sampling procedure in the anal canal. Aiming at

the improvement of this procedure, the College of American Pathologists (CAP) recommends the ampliation of its use.<sup>2</sup>

In this study we report the performance of this test in the presence of macroscopic lesions in the anal canal in outpatients seen at the Hospital Federal de Ipanema/MS/Rio de Janeiro, using the conventional method, a technology widely available in our country. Should it prove appropriate, it will contribute for the prevention of anal canal cancer in outpatient visits.

## Material and method

From April 2005 to December 2011, 395 cytopathology exams of anal canal were compared with the clinical diagnosis of presence of macroscopic lesions. The information was extracted from cytologic requisitions and medical records of the patients examined.

The study was approved by the Ethics Committee on Human Research of the Hospital Federal dos Servidores do Estado (CEP\_HFSE - 000474 protocol).

Patients whose samples were included in this trial were mostly men attended at our proctology outpatient clinic. The reasons of the visits were anal sex practice, some anal claim arising out of sexually transmitted disease or otherwise, presence of warts, or seropositivity for HIV. Samples of three patients from the gynaecology outpatient clinic were also included. One of them had perianal condylomata and the other two a diagnosis of vulvar intraepithelial neoplasia (VIN II and VIN III).

In most cases, the sampling was done in two slides at the first consultation, because no prior preparation is necessary. Patients who reported having receptive anal sexual relationship or made use of suppositories, creams or enema the day before the consultation were told to return later, observing the necessary precautions before collection.

As for the collection, two clean glass slides with frosted end, with the initials and clinical record number of the patient, and an unplugged slide rack tube containing ethyl alcohol up to 1 cm from the edge were arranged on a auxiliary table. The patient was placed in the Sims (left lateral decubitus position, with legs tucked) or gynaecological position. The anal slit was then opened with the left hand of the operator, and the patient was requested to help in the case of bulky buttocks, to prevent contamination with external condylomata, when present. With the right hand, the operator introduced a cytobrush previously moistened with distilled water or physiological saline for 4-5 cm, in order to reach the anal transformation zone starting 1 cm below the pectineal line and extending up to 2 cm above its upper edge. Then, soft and slow rotation movements were performed, pressing lightly on the wall of the anal canal and lower rectum, and the collection was carried out blindly.

The cytobrush containing the collected material was then rotated with a uniform motion on the central part of each slide, resulting in thin smears arranged longitudinally that, within 10 seconds and still wet, were totally immersed in hydrated ethyl alcohol for fixation of the samples (Fig. 1).

After collection, an inspection with conventional touch and anoscopy was performed. In patients older than 50 years a rectosigmoidoscopy was also performed. Then, the samples were sent for the Pathology Service, along with the application form containing clinical data; there, the slides were stained by Papanicolaou. The reports were issued by an experienced cytopathologist reporting the representativeness of epithelia present in the sample and the diagnostics, based on cytomorphological criteria for diagnostic elaboration according to The Bethesda System for Reporting Cervical Cytology<sup>8</sup> (Fig. 2). Considering that the conventional method was used, the criteria for the adequacy of the sample were based on the 1991 version of The Bethesda System, which defines the sample as "paucicellular"



Fig. 1 – Appearance of adequate Pap smears after staining.

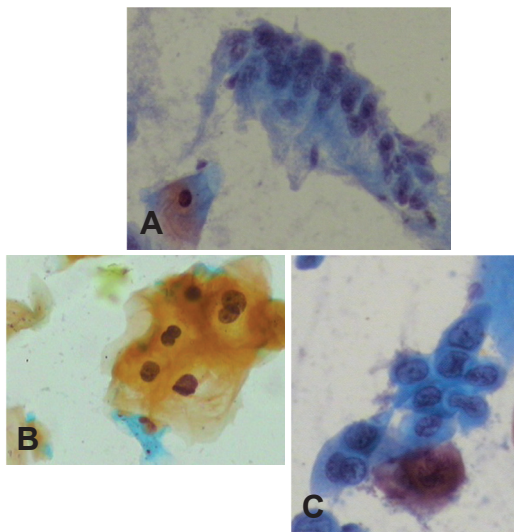


Fig. 2 – A, Columnar cells from the anal transformation zone. B, Low-grade anal intraepithelial lesion. C, High-grade anal intraepithelial lesion. (Conventional cytology stained by Papanicolaou method  $\times 400$ .)

when it covers less than 10% of the surface of the slide, and "prejudiced" when fixation artefacts, overlapping, blood stains and other contaminants affect 50-75% of the sample.

Samples composed exclusively of keratinized squamous cells; paucicellular samples; or those with artefacts of fixation, overlapping, exudate, erythrocytes and faecal material that prevented the diagnosis were judged unsatisfactory.

Data were stored in spreadsheets and analyzed using SPSS v. 21.

## Results

The sample consists of 395 cytological exams of patients predominantly male, and, among these, of HIV-seropositive ones (59.7%). Most patients (61.2%) presented lesions at the time of collection. The injury to the anal canal, visualized by conventional anoscopy, was present in 31.7% of men and in 22.5% women (Table 1).

At the moment of the collection, the clinical inspection of the perianal region and a conventional anoscopy showed predominance of macroscopic lesions in HIV-positive patients (62.5%) (data not shown).

Most of the samples were judged satisfactory by evaluation according to the criteria defined by Bethesda (Table 2). The most frequent cause of limitation of samples was pauci-

**Table 1 – Characteristics of patients from whom the specimens for cytopathological exam of anal canal (HFI/MS, 2005-2011) were obtained (cytobrushing).**

| Characteristics                          | Male         | Female       | Total        |
|--|--------------|--------------|--------------|
| Mean age in years (range)                | 36.8 (16-84) | 41.9 (21-80) | 37.9 (16-84) |
| HIV situation                            |              |              |              |
| HIV+                                     | 188 (59.7%)  | 15 (18.7%)   | 203          |
| HIV -                                    | 64 (20.3%)   | 29 (36.2%)   | 93           |
| HIV unknown                              | 63 (20%)     | 36 (45%)     | 99           |
| Presence of macroscopic lesion           |              |              |              |
| Perianal                                 | 102 (32.3%)  | 22 (27.5%)   | 124          |
| Anal canal                               | 45 (14.3%)   | 15 (18.7%)   | 60           |
| Both                                     | 55 (17.6%)   | 3 (3.8%)     | 58           |
| Absence of perianal or anal canal lesion | 113 (35.8%)  | 40 (50%)     | 153          |
| Total                                    | 315          | 80           | 395          |

**Table 2 – Classification of the adequacy of smears obtained by anal cytobrushing (HFI/MS, 2005-2011).**

| Smear characteristics               | Male        | Female   | Total       |
|-------------------------------------|-------------|----------|-------------|
| Satisfactory samples <sup>a</sup>   | 292 (92.6%) | 70 (87%) | 362 (91.6%) |
| Unsatisfactory samples <sup>b</sup> | 6 (60%)     | 4 (40%)  | 10 (2.5%)   |
| Limited samples <sup>c</sup>        | 17 (73.9%)  | 6 (26%)  | 23 (5.8%)   |
| Total                               | 315         | 80       | 395         |

<sup>a</sup>Representative and preserved cellularity, composed of keratinized and non-keratinized squamous cells and/or transitional and/or columnar cells. <sup>b</sup>Unrepresentative cellularity, consisting exclusively of keratinized squamous cells. <sup>c</sup>Limited cellularity, with poor fixation, presence of blood, exudate or faecal material.

cellularity (43.4%) followed by exudate, bacteria and hematic background. The artefacts of fixation (13%) and faecal material presence (4.3%) had low significance.

The analysis of the frequency of unsatisfactory samples over time demonstrates a downward trend, but the same was not observed with respect to the limited samples. There was an increase in this category in 2011, due to paucicellularity and exudate. The downward trend in the proportion of inadequate samples was statistically significant (linear regression,  $p = 0.048821$ ) (Table 3).

Representative elements of the anal transformation zone in 63.5% of samples were found, with no significant difference between genders (data not shown).

Among the tests considered satisfactory and altered, a predominance of low-grade lesions was perceived, especially in men; but it is worth mentioning a significant percentage of diagnoses of major relevance (Table 4).

The analysis of the diagnostic performance of anal cytology for detection of macroscopic lesions of the anal canal showed significant increase in sensitivity when the collection brought representative elements of the anal transformation zone (Table 5).

## Discussion

The prevention of anal cancer in Brazil is critical. Information campaigns are essential to reach a population exceeding 1.5 million MSM, according to a survey conducted on attitudes and practices in the Brazilian population in 2008 (PCAP-BR)<sup>9</sup> and women with a history of genital intraepithelial neoplasia.<sup>10</sup>

The methodology of liquid medium is still incipient among us and the biased and critical view of the conventional method, which is widely available, has resulted in few services performing exfoliative cytology of the anal canal.

The use of this method in services that do not have an anoscope, including those providing care for HIV+ patients, should be encouraged to evaluate the presence of injury and to referral of patients with abnormal tests for centres with adequate equipment.<sup>11</sup> Protocols and diagnostic algorithms based on cytologic changes are already suggested, in order to reduce the incidence of anal cancer in high-risk populations.<sup>12</sup>

The intent of this study was to evaluate the performance of conventional exfoliative cytology in the diagnosis of macroscopic lesions in the anal canal. We also analyzed the procedure as a function of sample quality. The lack of information about subclinical lesions prevents a more accurate analysis of this performance, but many patients remain continue to be followed-up, having been submitted to high-resolution anoscopy and biopsies, both liable to assessment in a next step.

The reduced percentage of unsatisfactory samples in this study is similar to the 6% reported by Mathews WC et al.,<sup>13</sup> while studying 2,947 conventional smears of the anal canal. We believe that the training given to proctologists, coupled with the fact that the same physician collected most samples (78%), has contributed to our results, being an essential step in implementing the use of this practice in a health care facility. On the other hand, Sherman et al.,<sup>14</sup> after months of training, considered most of conventional samples unsatisfactory, attributing the failure to the fact that the professionals involved in the collection, both medical and nonmedical, had no prior experience in collecting this type of sample.

**Table 3 – Evolution of the percentage of inadequate samples in smears obtained from anal cytobrushing over the years of study (HFI/MS, 2005-2011).**

|                              | 2005  | 2006 | 2007  | 2008  | 2009 | 2010  | 2011  | Total |
|------------------------------|-------|------|-------|-------|------|-------|-------|-------|
| Number of exams              | 14    | 22   | 68    | 57    | 52   | 81    | 101   | 395   |
| Unsatisfactory samples       | 3     | 1    | 5     | 0     | 0    | 1     | 0     | 10    |
| Limited samples              | 0     | 1    | 9     | 4     | 3    | 1     | 5     | 23    |
| Total of inadequate samples* | 3     | 2    | 14    | 4     | 3    | 2     | 5     | 33    |
| %                            | 21.4% | 9.0% | 20.5% | 14.0% | 5.7% | 2.46% | 4.95% |       |

\* Result of the sum of unsatisfactory and limited samples.

**Table 4 – Cytopathological diagnoses of smears considered as satisfactory, obtained by anal cytobrushing (HFI/MS, 2005-2011).**

| Cytopathological diagnosis                                      | Male        | Female     | Total       |
|---|-------------|------------|-------------|
| Negative  | 166 (56.8%) | 51 (72.8%) | 217 (59.9%) |
| Atypical squamous cells, possibly non neoplastic. ASC-US        | 37 (12.7%)  | 7 (10%)    | 44 (12.1%)  |
| Atypical squamous cells, not to discard high-grade lesion ASC-H | 4 (0.13%)   | 0 (0%)     | 04 (1.1%)   |
| Low-grade anal intraepithelial lesion                           | 69 (23.6%)  | 8 (11.4%)  | 77 (21.3%)  |
| High-grade anal intraepithelial lesion                          | 15 (5.1)    | 4 (5.7%)   | 19 (5.2%)   |
| Carcinoma   | 1 (0.34)    | 0 (0%)     | 01 (0.3%)   |
| TOTAL   | 292         | 70         | 362         |



**Table 5 – Diagnostic performance of cytopathologic exam of smears considered satisfactory, obtained by anal cytobrushing, in the detection of macroscopic lesions of anal canal (HFI/MS, 2005-2011).**

|  | Macroscopic lesion of<br>anal canal present | Macroscopic lesion of<br>anal canal absent | Total | S <sup>a</sup> | E <sup>b</sup> | RP <sup>c</sup> |
|--|---|--|-------|----------------|----------------|-----------------|
| <i>In satisfactory cytological samples</i>                                     |   |  |       |                |                |                 |
| Altered cytology   | 68  | 77   | 145   | 64%            | 69%            | 2.06            |
| Normal cytology  | 38  | 179  | 217   |                |                |                 |
| Totals   | 106   | 256  | 362   |                |                |                 |
| <i>In satisfactory cytological samples with elements of ZTA<sup>d</sup></i>    |   |  |       |                |                |                 |
| Altered cytology   | 57  | 61   | 118   | 80%            | 60%            | 2.0             |
| Normal cytology  | 14  | 98   | 112   |                |                |                 |
| Totals   | 71  | 159  | 230   |                |                |                 |
| <i>In satisfactory cytological samples without elements of ZTA<sup>d</sup></i> |   |  |       |                |                |                 |
| Altered cytology   | 11  | 16   | 27    | 31%            | 16%            | 0.37            |
| Normal cytology  | 24  | 81   | 105   |                |                |                 |
| Totals   | 35  | 97   |       |                |                |                 |

<sup>a</sup>Sensitivity; <sup>b</sup>specificity; <sup>c</sup>odds ratio positive; <sup>d</sup>anal transformation zone.

Our experience with anal canal cytology began in 2005 with the examination of smears obtained from patients included in this study, when several studies had already described the differences between cytological samples of cervical and anal canal.<sup>7,14-16</sup> One of our concerns was to meet the criteria contained in The ABCs of anal-rectal cytology CAP May 2004,<sup>17</sup> limiting the inconclusive diagnoses of atypical cells of undetermined significance (ASC-US and ASC-H).

The works de Ruiter et al.<sup>18</sup> and Palefsky et al.<sup>19</sup> correlated the representativeness of the anal transformation zone in the conventional sample with diagnostic performance and presence of lesions in the anal canal. In the first trial, 154 conventional cytological samples versus microanatomy-guided histological diagnoses of anal biopsy were evaluated, and the presence of anal transformation zone cells was considered as a criterion of adequacy. These authors found a sensitivity of 87.5% and a specificity of 16.3%.

Palefsky et al.<sup>19</sup> evaluated 658 MSM, and most remained in follow-up for two years. These authors correlated the conventional cytopathological exam with high-resolution anoscopy and histopathology. The results were grouped according HIV status and representativeness of the anal transformation zone in the sample. The HIV-positive participants had sensitivity of 69% and specificity of 59%; and the seronegative participants, sensitivity of 47% and specificity of 92%. Palefsky et al. concluded that the absence of columnar cells did not affect the diagnosis.

The study showed discrepant results when comparing the samples with and without components of the anal transformation zone, in disagreement with the opinion of some authors, who consider the presence of cells of the anal transformation zone as a qualitative data, without impact on the result. However, recent review articles point out that there are few publications addressing the minimum standard of adequacy of anorectal samples, and how the cellularity influences the sensitivity and specificity of the method.<sup>1,2</sup>

Anal cancer is related to a natural body orifice, and therefore its diagnosis should be easy and established early. However, the fact that is the anus, with all its burden of stigma and prejudice, coupled with the fact that its symptoms are indistinguishable from symptoms of the most common anal

diseases, contributes for diagnoses often obtained at more advanced clinical stages and not always of easy solution.<sup>20</sup>

The recognition of the risk of developing anal cancer is still undervalued by physicians and even by those social groups whose patients of the so-called risk group have visibility.<sup>2</sup>

## Conclusion

For the authors, this study demonstrated that the use of the conventional cytology method, widely available in our country, is a viable technology, provided that all the steps of collecting and fixing the samples be performed carefully, including allowing their transport when the samples are taken at distant locations from the laboratory.

## Conflicts of interest

The authors declare no conflicts of interest.

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